

- (21) Application No. 11959/72 (22) Filed 15 March 1972  
 (31) Convention Application No. 1626871 (32) Filed 17 March 1971 in (19)  
 (33) Russia (SU)  
 (44) Complete Specification published 5 June 1974  
 (51) International Classification C12D 9/14 C07G 11/00  
 (52) Index at acceptance

C2A 1B 1C1D1 2A3

- (72) Inventors GEORGY FRANTSEVICH GAUZE  
 TATYANA PAVLOVNA PREOBRAZHENSAYA  
 TATYANA SEMENOVNA MAXIMOVA  
 ROZA SERGEEVNA UKHOLINA  
 NATALIA PETROVNA NECHAEVA and  
 IYA NIKOLAEVNA KOVSHAROVA



## (54) A METHOD FOR PREPARING HELIOMYCIN

(71) We, VSESOJUZNY NAUCHNO-  
 ISSLEDOVATELSKY INSTITUT PO  
 IZYSKANIJU NOVYKH ANTIBIOTIKOV  
 AKADEMII MEDITSINSKIKH NAUK  
 5 SSSR, 11, Bolshaya Pirogovskaya ulitsa,  
 Moscow, Union of Soviet Socialist Republics  
 (U.S.S.R.), A State Enterprise organised and  
 existing under the laws of U.S.S.R., do hereby  
 declare the invention, for which we pray that  
 10 a patent may be granted to us, and the method  
 by which it is to be performed, to be partic-  
 ularly described in and by the following  
 statement:—

This invention relates to a method of pre-  
 15 paring the antibiotic heliomycin which may  
 be used as a 4 per cent ointment for treating  
 burns, various forms of pyodermatitis and in  
 cosmetic medicine owing to its antibacterial  
 and vasoconstrictive action.

20 A method is known by which heliomycin is  
 produced by the culturing of *Actinomyces*  
*flavochromogenes* var. *heliomycini*. The start-  
 ing culture is grown by the submerged tech-  
 25 nique in a medium containing (in per cent by  
 weight) soya bean flour, 1.0; starch, 5—2.0;  
 primary potassium phosphate, 0.1; sodium  
 chloride, 0.3; calcium carbonate, 0.3; sperm  
 oil, 0.1; water to make 100 per cent.

30 The same culture medium can be used for  
 growing the seeding mycelium.

The mycelium is separated from the culture  
 fluid by filtering and the antibiotic is then  
 extracted from the mycelium with acetone.  
 35 The moisture content of the mycelium is about  
 60 per cent by weight. The extraction is re-  
 peated four times, taking three litres of acetone  
 per kilogram of damp mycelium. The first ex-  
 tract is discarded as having low potency, while  
 40 the second and the third extracts are mixed  
 with a double volume of distilled water. The  
 fourth extract is used for the second extrac-  
 tion of mycelium obtained in the next fer-  
 mentation.

[Price 25p]

The resulting crystalline precipitate of helio-  
 mycin is separated on a filter and washed with  
 45 distilled water. If the crude product contains  
 oil from the medium, it will dry with diffi-  
 culty. Hence another wash with petroleum  
 ether is required.

The yield of crude heliomycin is three per  
 50 cent by weight calculated with reference to  
 moist mycelium. The crude preparation con-  
 tains from 80 to 85 per cent by weight of  
 heliomycin.

The disadvantage of the known method for  
 55 preparing heliomycin by the culture of *Actino-*  
*myces flavochromogenes* var. *heliomycini* is  
 the great variability of the strain. During the  
 frequent re-seeding and lengthy storage, the  
 culture develops into more productive and less  
 60 productive variants. The greatest activity with  
 respect to accumulating the antibiotic in the  
 mycelium is inherent in the variants whose  
 colonies, when grown on a solid organic  
 medium, have no aerial mycelium, and when  
 65 grown on a mineral medium, have but meagre  
 aerial mycelium and liberate reddish brown  
 pigment. For these reasons the yield of the  
 antibiotic is low (3 per cent by weight with  
 70 reference to the moist mycelium, even with  
 the utilization of active variants of the cul-  
 ture *Actinomyces flavochromogenes* var. *heli-*  
*omycini*).

An object of the present invention is to  
 75 obviate or mitigate the disadvantages of the  
 aforesaid method.

According to the present invention there is  
 provided a method of preparing the anti-  
 biotic heliomycin, comprising aerobically cul-  
 80 tivating a heliomycin-producing strain of  
*Actinomyces variabilis* var. *roseolus* in an  
 aqueous, nutrient medium containing assimil-  
 able sources of nitrogen, carbon and mineral  
 salts nutrient medium separating the thus  
 85 formed mycelium and extracting the helio-  
 mycin therefrom.

- The new strain of *Actinomyces variabilis* var. *roseolus*, is characterized by the following physiological and morphological properties: when grown on Hause's mineral medium No. 1 it produces ample aerial mycelium of grey colour; the substrate mycelium is colourless; the medium is coloured faint pinkish brown or violet-brown; the sporangiophores are spiral, the spirals having 2—4 coils and the spores are covered with long pili; when grown in Hause's organic medium No. 2, the aerial mycelium is first creamy in colour, then it turns grey, while the substrate mycelium is from yellowish brown to dark brown in colour; when grown in Czapek and Lindenbein's medium, the aerial mycelium is ample and grey in colour, the substrate mycelium is brown; on glucose-asparagine medium the aerial mycelium is scarce, first creamy in colour, then grey; the substrate mycelium is yellowish brown, the medium is colourless; on liquid nutrient media at temperatures of 28°C it readily assimilates lactose, galactose, moderately assimilates glucose and poorly assimilates dulcitol. The strain is isolated from soil.
- Any embodiment of the present invention will now be described by way of illustration.
- Strain No. 6383 of *Actinomyces variabilis* var. *roseolus* which is used as the starting culture, differs from strain No. 2915 of *Actinomyces flavochromogenes* var. *heliomycinii* in that it forms brown substrate mycelium and soluble pigments (pinkish brown or violet brown) when grown on synthetic media, and also in that ample quantities of spores are borne by the aerial mycelium grown on these media.
- The starting culture is grown submerged in a soya-starch medium. The same medium can also be used for the productive fermentation.
- The antibiotic is contained in the mycelium, which is separated from the culture fluid by filtration. The antibiotic is extracted with acetone from the mycelium which contains about 60 per cent by weight of moisture. The mycelium is extracted four times each with three litres of acetone per kilogram of damp mycelium. The potency of the first extract is low and it is therefore discarded. The second and the third extracts are combined and then mixed with twice their volume of distilled water to precipitate the heliomyces.
- The fourth extract is used for the second extraction of mycelium from the next fermentation. The precipitated crystals of heliomyacin are separated on a filter and washed with distilled water. If the crude antibiotic contains oily compounds it dries with difficulty and should therefore be given an additional wash with petroleum ether. The yield of crude antibiotic is 4 per cent by weight calculated with reference to the damp mycelium.
- The crude preparation contains 80 to 85 per cent by weight of heliomyacin calculated with reference to a chemically pure crystalline preparation.
- The advantage of the proposed method lies in the utilization of a new and more productive micro-organism, viz., the strain No. 6383 of *Actinomyces variabilis* var. *roseolus*, which provides a yield of crystalline heliomyacin of up to 4 per cent by weight with respect to moist mycelium.
- Example
- The spores are prepared by growing strain No. 6383 of *Actinomyces variabilis* var. *roseolus* in test tubes on a synthetic Hause's medium No. 1 slant for ten days at a temperature of 28°C. The inoculum is prepared by growing the spores in 500 ml Erlenmeyer flasks containing 100 ml of culture medium of the following composition, in per cent by weight:
- |                             |                |    |
|-----------------------------|----------------|----|
| soya bean flour             | 1.0            | 85 |
| starch                      | 1.0            |    |
| sodium chloride             | 0.3            |    |
| calcium carbonate           | 0.03           |    |
| primary potassium phosphate | 0.1            |    |
| water                       | to make 100 ml | 90 |
- The pH of the medium after sterilization is 7.0.
- The seeding mycelium is grown in the flasks on reciprocating shakers (200 rpm) for two days at a temperature of 28°C.
- Then the inoculum (5 per cent by weight, or 400 ml per tank) is introduced into seeding tanks of 45 litre capacity containing 20 litres of nutrient medium of the following composition, in per cent by weight:
- |                             |                |     |
|-----------------------------|----------------|-----|
| soya bean flour             | 1.0            |     |
| starch                      | 1.0            |     |
| sodium chloride             | 0.3            |     |
| calcium carbonate           | 0.3            |     |
| primary potassium phosphate | 0.1            | 105 |
| sperm oil                   | 0.1            |     |
| water                       | to make 100 ml |     |
- The pH of the medium after sterilisation is 7.0.
- The medium before inoculation has been sterilised for 45 minutes at a temperature of 120°C. The culture is fermented at a temperature of 28°C, a pressure of 0.3 to 0.5 atm, and an aeration rate of one litre of air per litre of the medium per minute. The contents are stirred continually at 300 rpm.
- The fermentation is continued for 48 hours and the grown culture is discharged into 500-lit fermentation tanks to inoculate the nutrient

medium of the following composition, in per cent by weight:

	soya bean flour	1.0
	starch	2.0
5	sodium chloride	0.3
	calcium carbonate	0.3
	primary potassium phosphate	0.1
	sperm oil	0.1
	water	to make 100

- 10 The pH of the medium after sterilization is 7.0.  
The medium is sterilized for 43 minutes at a temperature of 120°C. The fermentation tanks are inoculated with 20 litres of the seeding material per 300 litres of the culture medium, which is about 5 to 7 per cent by weight of the seeding material.
- 15 The fermentation is carried out at a temperature of 28°C and a pressure of 0.3 to 0.5 atm. The air is sparged through the liquid at a rate of one litre per litre of medium per minute. The contents are continually stirred at 300 rpm.
- 20 The fermentation is continued for 96 to 120 hours.
- 25 The 300 litres of the fermented broth are passed through a filter to separate about 30 kg of moist mycelium containing about 60 per cent by weight of moisture.
- 30 The mycelium is extracted with acetone four times. Each time the volume of the acetone is three times (90 litres) the weight (30 kg) of the moist mycelium. The first extract having a low potency is discarded, and the second and the third extracts are combined and mixed with twice their volume of distilled water (360 litres). The resulting precipitated crystalline heliomycin is separated on a filter, and washed with distilled water. If the crude antibiotic contains oily impurities, it dries with difficulty and therefore it is given an additional wash with petroleum ether, in an amount of 5 to 10 volumes with respect to the crystalline crude antibiotic.
- 40 The 30 kg of moist mycelium (60 per cent by weight of moisture) yield 1,200 g of crude antibiotic containing from 80 to 85 per cent by weight of heliomycin, calculated with respect to chemically pure crystalline heliomycin.
- 50

#### WHAT WE CLAIM IS:—

1. A method of preparing the antibiotic heliomycin, comprising aerobically cultivating a heliomycin-producing strain of *Actinomyces variabilis* var. *roseolus* in an aqueous nutrient medium containing assimilable sources of nitrogen, carbon and mineral salts, separating the thus formed mycelium and extracting the heliomycin therefrom. 55
2. A method as claimed in claim 1, wherein the strain of *Actinomyces variabilis* var. *roseolus* is characterised by the following physiological and morphological properties: when grown on Hause's mineral medium No. 1 it produces ample aerial mycelium of grey colour; the substrate mycelium is colourless; the medium is coloured faint pinkish brown or violet-brown; the sporangiophores are spiral, the spirals having 2—4 coils and the spores are covered with long pili; when grown in Hause's organic medium No. 2, the aerial mycelium is first creamy in colour, then it turns grey, while the substrate mycelium is from yellowish brown to dark brown in colour; when grown in Czapek and Lindenbein's medium, the aerial mycelium is ample and grey in colour, the substrate mycelium is brown; on glucose-asparagine medium the aerial mycelium is scarce, first creamy in colour, then grey, the substrate mycelium is yellowish brown, the medium is colourless; on liquid nutrient media at temperatures of 28°C it readily assimilates lactose and galactose, moderately assimilates glucose and poorly assimilates dulcitol. 60 65 70 75 80 85
3. A method for preparing heliomycin according to claim 1, substantially as hereinbefore described and with reference to the Example.
4. The antibiotic heliomycin whenever prepared by the method as claimed in any one of the preceding claims. 90

FITZPATRICKS,  
Chartered Patent Agents,  
14—18, Cadogan Street,  
Glasgow, G2 6QW,  
and  
Warwick House,  
Warwick Court,  
London, WC1R 5DJ.